Endokrynologia Polska
DOI: 10.5603/EP.2014.0025
Tom/Volume 65; Numer/Number 3/2014
ISSN 0423–104X

The influence of endocannabinoid receptor 1 gene variations on anthropometric and metabolic parameters of women with polycystic ovary syndrome

Wpływ polimorfizmu genu receptora endokanabinoidowego 1 na parametry antropometryczne i metaboliczne u kobiet z zespołem wielotorbielowatych jajników

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Abstract

Introduction: Polycystic ovary syndrome (PCOS) is associated with an increasing number of metabolic comorbidities. About 50% of PCOS patients are obese, and insulin resistance affects up to 70% of these women. The endocannabinoid system contributes to human energy homeostasis. CNR1 is a biological candidate for human obesity and related metabolic disorders. The aim of this study was to determine the relationships between CNR1 polymorphisms and anthropometric and metabolic parameters in PCOS women.

Material and methods: 130 women diagnosed with PCOS according to the Rotterdam criteria were recruited. The control group consisted of 70 healthy women. Medical history was taken, and physical examination as well as assessment of anthropometric (body mass, height, waist and hip circumference, BMI, waist-to-hip ratio [WHR]) and metabolic parameters (glucose and insulin, the insulin resistance index HOMA, lipid profile) was carried out. Genetic studies to detect six CNR1 gene polymorphisms were performed.

Results: The total cholesterol and low-density lipoprotein (LDL) cholesterol levels in PCOS women carrying T/T genotype of rs2023239 CNR1 polymorphism were higher than in those with C/T and C/C. There were no statistical differences in other metabolic parameters or in the value of BMI and WHR between the variants of rs2023239 CNR1 polymorphism. The other studied polymorphisms of the CNR1 gene were not associated with anthropometric or metabolic parameters in PCOS women. There were no differences in anthropometric or metabolic parameters between the variants of studied polymorphisms of the CNR1 gene in control women.

Conclusions: On the basis of our study, it seems that CNR1 polymorphisms are not associated with obesity and metabolic disorders, including insulin resistance, in PCOS women. **(Endokrynol Pol 2014; 65 (3): 181–188)**

Key words: *polycystic ovary syndrome; endocannabinoid system; CNR1 polymorphism*

Streszczenie

Wstęp: Zespół wielotorbielowatych jajników wiąże się z licznymi zaburzeniami metabolicznymi. Około 50% kobiet z PCOS jest otyłych, a insulinooporność wykazuje do 70% kobiet z tym zespołem. Układ endokanabinoidowy odgrywa rolę w regulacji równowagi energetycznej organizmu. Gen CNR1 jest genem kandydatem związanym z otyłością i zaburzeniami metabolicznymi. Celem badania była ocena wzajemnych powiązań między polimorfizmami genu CNR1 a parametrami antropometrycznymi i metabolicznymi u pacjentek z PCOS.

Materiał i metody: Do badania włączono 130 kobiet, u których w oparciu o kryteria rotterdamskie postawiono rozpoznanie PCOS. Grupę kontrolną stanowiło 70 zdrowych kobiet. U wszystkich badanych przeprowadzono wywiad lekarski, badanie fizykalne z oceną parametrów antropometrycznych (masa i wysokość ciała, obwód talii i bioder, indeks masy ciała, wskaźnik talia–biodra), wykonano badania biochemiczne (ocena stężeń glukozy i insuliny, wskaźnika insulinooporności HOMA, lipidogramu). Przeprowadzono również badania genetyczne oceniające sześć polimorfizmów genu CNR1.

Wyniki: Stężenie cholesterolu całkowitego i cholesterolu frakcji LDL było wyższe u kobiet z PCOS i genotypem T/T polimorfizmu rs2023239 genu CNR1 w porównaniu z pacjentkami z genotypami C/T i C/C. Nie było istotnych statystycznie różnic dotyczących innych parametrów metabolicznych (stężenia glukozy, insuliny, wskaźnik HOMA) ani wskaźników masy ciała i talia–biodra między poszczególnymi genotypami polimorfizmu rs2023239. Nie wykazano zależności pomiędzy innymi badanymi polimorfizmami genu CNR1 a parametrami antropometrycznymi i metabolicznymi u pacjentek z PCOS. Nie stwierdzono również takich zależności u kobiet z grupy kontrolnej.

Wnioski: Na podstawie przedstawionego badania wydaje się, że polimorfizmy genu CNR1 nie są związane z otyłością ani zaburzeniami metabolicznymi, w tym insulinoopornością, u kobiet z PCOS. **(Endokrynol Pol 2014; 65 (3): 181–188)**

Słowa kluczowe: *zespół wielotorbielowatych jajników; układ endokanabinoidowy; polimorfizm genu CNR1*

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Introduction

Polycystic ovary syndrome (PCOS) is among the most commonly diagnosed endocrine diseases in women of reproductive age. It is characterised by hyperandrogenism, abnormalities in gonadotropin secretion, chronic anovulation and polycystic ovaries [1, 2]. PCOS patients have more metabolic disturbances than the general population and are at increased risk of developing type 2 diabetes [3–5] and cardiovascular disease [6]. About 50% of PCOS patients are obese, while insulin resistance and hyperinsulinaemia affect up to 70% of women with this syndrome [7]. Abnormalities in carbohydrate metabolism in PCOS patients include abnormal glucose tolerance as well as type 2 diabetes [8] and in lipid metabolism decreased levels of high-density lipoprotein (HDL) cholesterol and increased LDL cholesterol and triglycerides [9]. The prevalence of metabolic syndrome in patients with PCOS ranges from 33.4% to 47%. There is evidence of an increased incidence of hypertension in PCOS patients [10–12].

The endocannabinoid system contributes to human energy homeostasis and plays a role in lipid and carbohydrate metabolism and also in fat accumulation in liver and muscles [13–16]. Its key factors are the cannabinoid receptors that are expressed in a variety of tissues including the adipose tissue, liver, skeletal muscle, brain, gastrointestinal tract and pancreas [17]. The cannabinoid receptor 1 gene (CNR1), which encodes the cannabinoid receptor, is a biological candidate for human obesity and related metabolic disorders and is located on chromosome 6 (6q14-q15). There is evidence about the higher activation of the endocannabinoid system in obese people and animals and higher expression of CNR1 in visceral adipose tissue [18]. There are some studies investigating the relationships between common variants of CNR1 and obesity as well as metabolic disturbances, but the results of these studies are conflicting. In one study, carriers of 3813G variant of CNR1 had central distribution of fatty tissue and general high accumulation of adipose tissue [19,20]. In another study, A3813G polymorphism was related to higher waist circumference and G1422A polymorphism was associated with higher waist circumference and higher value of waist-to-hip ratio [21]. In our previous study, we found relationships between various CNR1 polymorphisms and obesity in postmenopausal women [22]. However, there have also been some studies finding no associations between CNR1 polymorphisms and obesity [23–26].

To date, there is no evidence about the CNR1 polymorphisms in women with PCOS. These correlations are especially interesting because metabolic disturbances are common in PCOS women and, on the other hand, the endocannabinoid system not only plays a role in metabolic aspects of body weight, but also influences the hypothalamic-pituitary axis and function of gonads [27, 28]. Demonstration of the association between CNR1 polymorphisms and metabolic disorders could enable the early identification of women with PCOS particularly at risk of those metabolic abnormalities. Then the targeted therapy of medicines modifying the activity of the CNR1 receptor would be possible. The aim of our study was to investigate the influence of various CNR1 polymorphisms on anthropometric and metabolic parameters in PCOS women.

Material and methods

The study included 130 patients with PCOS (mean ± SD age: 24.91 ± 4.85 years, BMI: 26.96 ± 6.96 kg/m^2) diagnosed in the Department of Endocrinology, Diabetology and Isotope Treatment in the Medical University of Wroclaw. PCOS was diagnosed according to the Rotterdam consensus: two out of three criteria should be met: 1) oligomenorrhoea or amenorrhea (menstrual periods that occur at intervals of greater than 35 days or the absence of menstruation greater than six months); 2) clinical and/or biochemical hyperandrogenism (hirsutism on the Ferriman-Gallwey scale, score > 8 or high free androgen index > 7%); and 3) characteristic image of polycystic ovaries on gynaecological ultrasound (ovaries featuring either ten or more follicles measuring 2–9 mm in diameter or their volume greater than 10 cm^3). Patients with hypercortisolemia, hyperprolactinemia, impaired thyroid function, or suspicion of ovarian or adrenal tumour were excluded from the study. The control group consisted of 70 healthy women (mean ± SD age: 24.90 ± 4.25 years, BMI: 25.71 ± 6.27 kg/m^2). Patients eligible for the study stated that during the three months prior to the study they had not been on any special diet or practiced intense physical exercise, had consumed alcohol occasionally, and had smoked no more than five cigarettes a day. The study protocol was approved by the Ethics Committee of Wroclaw Medical University and all the subjects gave their informed consent in writing.

In all the subjects, the following were carried out:

— medical history and physical examination;
— assessment of anthropometric parameters (body mass, height, waist and hip circumference, BMI, WHR);
— evaluation of hormonal parameters (testosterone, sex hormone-binding globulin [SHBG], androstenedione, luteinising hormone [LH], follicle-stimulating hormone [FSH]);
— assessment of metabolic parameters (glucose, insulin, insulin resistance index HOMA, lipid profile);

— genetic studies to detect the various variants of the CNR1 gene.

Anthropometric measurements

Body weight and height were measured on a standard beam balance scale with an attached ruler. The waist circumference was measured at the umbilicus level and hip circumference at the level of the trochanter major to the nearest 0.1 cm with a flexible plastic tape. The body mass index (BMI) was calculated from the equation: weight [kg]/height [m^2], and the waist-to-hip ratio (WHR) as waist circumference [cm]/hip circumference [cm].

Biochemical measurements

Blood for laboratory tests was collected between 8:00 and 10:00 a.m. after overnight fasting, at least after eight hours after the last meal.

Serum concentrations of glucose, total cholesterol, HDL cholesterol, and triglycerides were measured by routine enzymatic methods (Dade Behring Marburg GmbH, Germany) and LDL cholesterol was calculated from Friedewald's formula: cholesterol LDL [mg/dL] = total cholesterol [mg/dL] – HDL cholesterol [mg/dL] – (triglycerides [mg/dL]/5).

Serum concentrations of insulin, testosterone, LH, FSH, and SHBG were measured by a radio-immunological method using commercial kits of DPC — Diagnostic Products Corporation, Los Angeles, CA, USA. The free androgen index (FAI) was calculated from the concentrations of total testosterone (T) and SHBG (FAI = T/SHBG × 100).

For the estimation of insulin resistance, the HOMA insulin resistance index: (insulin (μIU/mL) × glucose (mmol/L))/22.5 was computed (I_0 — fasting insulin, G_0 — fasting glucose). Subjects were considered as insulin resistant when the HOMA index was > 2.5.

Genetic studies

Whole genomic DNA was isolated from blood leukocytes using standard methods. *CNR1* genotyping (G1422A, A4895G and rs806381, rs10485170, rs6454674, rs2023239) was performed by two multiplex polymerase chain reactions (PCR) and minisequencing [29].

The first one: three fragments of the *CNR1* gene (347-bp, 346-bp, and 231-bp) were amplified using a multiplex PCR mix containing the specific three pairs of primers (Table I), 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 200 μM dTTP, 1× Q solution, two polymerase units (QIAGEN), 200 ng genomic DNA, and water for a total volume of 20 μL.

The DNA was denatured at 95°C for 3 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds.

To amplify the second group of the four fragments of the *CNR1* gene (205-bp, 230-bp, 280-bp and 304-bp), a multiplex PCR mix was used. It was employed containing the specific four pairs of primers (Table I), 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 200 μM dTTP, two hot-start polymerase units (TAKARA), 200 ng genomic DNA, and water for a total volume of 20 μL. The DNA was denatured at 95°C for 3 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 45 seconds.

The amplified fragments were purified from oligonucleotides and free dNTPs by SAP and ExoI treatment.

The minisequencing method was based on the incorporation of single fluorescence-labelled dideoxynucleotides to the 3′ end of the oligonucleotide that was correctly paired to the specific template DNA fragment using a SNaPshot kit (Applied Biosystems). Two SNaPshot reactions were carried out using the oligonucleotides:

— G1422A: 5′-TGCAGCCAGTGTTCACAGGGCCGCAGAAAGCTGCATCAAGAGCAC-3′
— A4895G: 5′-TTAAGATGCCACGGCAATGTAAAGAAACTCTCCCA-3′
— rs806381: 5′-TCCAACAAATGAGTGACCGTTACC-3′
— rs10485170: 5′-ACTAGAGTTGTGCTGAGTTAATACATGAGATC-3′
— rs6454674: 5′-CTTCTCCAAAATATTTCCTGGAATAAAAGAAGCAATAACT-3′
— rs2023239: 5′-GGGTGGGAGTTGAAAGGCAAAAGCTAGGTTTGTGGATGTGCCAGGACCA-3′
— designed so that it ended immediately before the polymorphic side. The SNaPshot reaction consisted of 25 cycles: denaturation at 96°C for 10 seconds, annealing at 50°C for 5 seconds, and extension at 60°C for 30 seconds. The product was analysed by an ABI 3100 sequencer (Applied Biosystems). Product size was calculated using GeneScan 4.1 (Applied Biosystems).

Statistical analysis

Means and standard deviations of some anthropometric and biochemical parameters were calculated for genotypes of the analysed CNR1 polymorphisms. To compare means between genotypes, ANOVA was used. The level of statistical significance was set at $p < 0.05$.

Results

Anthropometric and metabolic variables according to the CNR1 variants are presented in Tables II–VII.

Table I. *Sequences of the CNR1 primers*
Tabela I. *Sekwencje starterów CNR1*

	Polymorphism	Forward primer (3'-5')	Reverse primer (3'-5')
1	G1422A (rs1049353)	CCTGCGACACGCTTTCCGGA	CTGCCAGGGAGGCATCAGGC
2	A4895G (rs806368)	GAGACCACCCATATCATGCACACA	AACTCTGATCCCCAGTAGGCCTAG
3	rs806381	CATGAGCCATGAGGTTTTCT	CATTTGAAGGCCTGTAACTT
4	rs10485170	TTAACCAATG GTTCATCGTC	ATGTGGTTCTCAGGCATCAG
5	rs6454674	ATGGAGCCTGTCCTTTAGGT	TATCCAGGAATGCTGCAAAA
6	rs2023239	AATTGAATCCAACCACAGGT	AGTTAGGAACTTAGGTGACT

Table II. *Anthropometric and metabolic variables according to the A4895G (rs806368) CNR1 polymorphism*
Table II. *Antropometryczne i metaboliczne zmienne według A4895G (rs806368) polimorfizmu CNR1*

		G/G	A/G	A/A	p
BMI [kg/m^2]	PCOS	27.32 ± 7.14	27.04 ± 7.39	26.21 ± 4.95	0.918602
	CG	24.89 ± 6.20	26.59 ± 6.31	24.76 ± 4.99	0.579367
WHR	PCOS	0.82 ± 0.08	0.81 ± 0.07	0.85 ± 0.08	0.468638
	CG	0.80 ± 0.06	0.82 ± 0.07	0.85 ± 0.06	0.180554
Glucose mean [mg/dL]	PCOS	86.13 ± 8.14	86.53 ± 10.87	92.43 ± 13.16	0.244467
	CG	80.38 ± 8.00	83.14 ± 8.49	83.50 ± 9.47	0.402779
Insulin mean	PCOS	11.17 ± 8.52	9.62 ± 6.48	9.08 ± 3.24	0.547144
[μIU/mL]	CG	8.10 ± 6.01	11.31 ± 9.55	5.85 ± 4.31	0.181982
HOMA	PCOS	2.36 ± 1.89	2.11 ± 1.57	2.00 ± 0.79	0.727103
	CG	1.62 ± 1.24	2.42 ± 2.39	1.23 ± 0.96	0.161612
TC	PCOS	195.78 ± 36.53	193.54 ± 39.15	189.14 ± 23.54	0.879270
[mg/dL]	CG	179.30 ± 3386	170.62 ± 37.28	174.25 ± 7.37	0.630938
LDL-C	PCOS	111.81 ± 31.68	108.22 ± 32.73	121.71 ± 44.82	0.594266
[mg/dL]	CG	95.71 ± 3069	91.70 ± 30.20	99.50 ± 9.33	0.838456
HDL-C	PCOS	60.32 ± 17.87	63.49 ± 15.00	65.57 ± 28.82	0.570079
[mg/dL]	CG	66.93 ± 17.39	59.57 ± 12.50	57.00 ± 11.69	0.151413
TG	PCOS	116.61 ± 72.97	93.92 ± 53.67	119.57 ± 122.50	0.271901
[mg/dL]	CG	79.76 ± 49.84	91.67 ± 79.49	89.25 ± 51.17	0753350

The total cholesterol and LDL cholesterol levels in PCOS women carrying the T/T genotype of rs2023239 CNR1 polymorphism were higher than in those with C/T and C/C. There were no statistical differences in other metabolic parameters (HDL cholesterol, triglycerides, glucose, insulin and HOMA levels) or in the value of BMI and WHR between the variants of rs2023239 CNR1 polymorphism. No correlations were found between the other studied polymorphisms of the CNR1 gene and the anthropometric and metabolic parameters in PCOS women. There were no differences in anthropometric and metabolic parameters between the variants of studied polymorphisms of the CNR1 gene in control women.

Discussion

In recent years, more attention has been paid to the endocannabinoid system, which plays an important role in energy regulation of several metabolic pathways. It participates in food intake, energy balance, lipid and glucose metabolism [13–18]. It also influences the hypothalamic-pituitary axis by decreasing serum LH levels and modulating GnRH secretion. High expression of endocannabinoids in the ovaries may negatively influence ovulation [27, 28]. Taking into account the fact that PCOS is the most common endocrinopathy in women of reproductive age, and that this syndrome

Table III. *Anthropometric and metabolic variables according to the A1422G (rs1049353) CNR1 polymorphism*
Tabela III. *Antropometryczne i metaboliczne zmienne według A1422G (rs1049353) polimorfizmu CNR1*

		A/G	G/G	A/A	p
BMI [kg/m^2]	PCOS	27.94 ± 7.43	27.04 ± 6.98	24.86 ± 5.86	0.500137
	CG	24.92 ± 6.13	25.98 ± 6.52	24.04 ±4.38	0.659792
WHR	PCOS	0.84 ± 0.09	0.81 ± 0.07	0.82 ± 0.07	0.260343
	CG	0.81 ± 0.06	0.81 ± 0.06	0.80 ± 0.07	0.976758
Glucose mean	PCOS	87.18 ± 8.89	86.68 ± 10.07	84.62 ± 8.19	0.782885
[mg/dL]	CG	81.38 ± 7.35	81.53 ± 9.15	81.13 ± 6.36	0.991806
Insulin mean	PCOS	10.92 ± 7.55	10.61 ± 8.31	10.45 ± 6.99	0.976230
[μIU/mL]	CG	9.41 ± 5.66	8.80 ± 8.27	8.83 ± 7.23	0.954736
HOMA	PCOS	2.39 ± 1.73	2.26 ± 1.89	2.19 ± 1.60	0.924196
	CG	1.91 ± 1.24	1.83 ± 1.97	1.81 ± 1.52	0.984544
TC	PCOS	197.86 ± 34.03	193.86 ± 36.61	188.00 ± 50.73	0.736143
[mg/dL]	CG	176.86 ± 37.40	179.08 ± 34.89	161.63 ± 9.30	0,420093
LDL	PCOS	114.80 ± 31.46	109.64 ± 32.09	113.12 ± 48.76	0.730545
[mg/dL]	CG	94.24 ± 30.67	99.78 ± 29.88	72.63 ± 10.07	0.058949
HDL	PCOS	61.09 ± 18.20	62.73 ± 17.75	54.37 ± 13.83	0.444302
[mg/dL]	CG	65.86 ± 17.07	60.71 ± 14.52	75.00 ± 16.11	0.056465
TG	PCOS	117.93 ± 69.59	102.89 ± 73.09	112.75 ± 66.92	0.557230
[mg/dL]	CG	69.76 ± 52.07	94.95 ± 67.55	69.88 ± 25.90	0.239373

Table IV. *Anthropometric and metabolic variables according to the rs806381 CNR1 polymorphism*
Tabela IV. *Antropometryczne i metaboliczne zmienne w zależności od polimorfizmu rs806381 CNR1*

		G/G	A/G	A/A	p
BMI [kg/m^2]	PCOS	26.37 ± 6.56	27.015 ± 7.47	27.85 ± 7.97	0.307152
	CG	23.57 ± 4,51	25.67 ± 5.76	24.67 ± 6.45	0.698493
WHR	PCOS	0.83 ± 0.07	0.82 ± 0.08	0.79 ± 0.08	0.376088
	CG	0.79 ± 0.08	0.81 ± 0.06	0.79 ± 0.05	0.406902
Glucose mean	PCOS	86.00 ± 7.79	87.31 ± 11.57	85.54 ± 8.12	0.909260
[mg/dL]	CG	81.80 ± 8.89	80.03 ± 7.87	81.22± 8.37	0.826644
Insulin mean	PCOS	10.27 ± 6.51	10.54 ± 9.23	10.72 ± 7.91	0.997454
[μIU/mL]	CG	8.10 ± 5.90	9.35 ± 6.55	7.49 ± 5.27	0.542649
HOMA	PCOS	2.21 ± 1.46	2.29 ± 2.10	2.11 ± 1.74	0.989013
	CG	1.67 ± 1.26	1.89 ± 1.44	1.50 ± 1.08	0.556540
TC	PCOS	195.22 ± 34.20	194.73 ± 40.05	189.15 ± 36.59	0.956966
[mg/dL]	CG	163.80 ± 20.63	180.03 ± 36.99	172.04 ± 30.42	0.500047
LDL	PCOS	109.51 ± 32.94	114.07 ± 33.63	107.61 ± 37.34	0.899724
[mg/dL]	CG	86.80 ± 19.18	98.80 ± 28.73	90.26 ± 30.92	0.477881
HDL	PCOS	63.87 ± 17.75	59.07 ± 17.90	66.85 ± 18.97	0.408336
[mg/dL]	CG	59.40 ± 11.67	63.70 ± 15.64	67.35 ± 17.55	0.535297
TG	PCOS	105.39 ± 66.69	112.50 ± 84.120	94.23 ± 46.10	0.873601
[mg/dL]	CG	88.80 ± 45.82	82.73 ± 57.36	71.61 ± 33.29	0.632409

Table V. *Anthropometric and metabolic variables according to the rs10485170 CNR1 polymorphism*
Tabela V. *Antropometryczne i metaboliczne zmienne w zależności od polimorfizmu CNR1 rs10485170*

		A/A	A/G	G/G	p
BMI [kg/m^2]	PCOS	27.48 ± 7.27	24.56 ± 6.50	22.37 ± 2.43	0.206867
	CG	25.02 ± 5.79	26.00 ± 6.64	19.29 ± 0.00	0.553472
WHR	PCOS	0.82 ± 0.08	0.81 ± 0.07	0.76 ± 0.01	0.533411
	CG	0.80 ± 0.06	0.80 ± 0.05	0.81 ± 0.00	0.935797
Glucose mean	PCOS	87.13 ± 10.08	84.41 ± 6.95	84.50 ± 4.95	0.541845
[mg/dL]	CG	81.45 ± 7.99	76.80 ± 7.96	81.45 ± 0.00	0.252519
Insulin mean	PCOS	10.39 ± 6.66	10.69 ± 12.42	7.65 ± 3.46	0.876319
[μIU/mL]	CG	8.13 ±15.93	10.94 ± 6.37	5.10 ± 0.00	0.375709
HOMA	PCOS	2.23 ± 1.54	2.27 ± 2.72	1.57 ± 0.63	0.871909
	CG	1.67 ± 1.31	2.05 ± 1.24	1.03 ± 0.00	0.630826
TC	PCOS	196.58 ± 37.39	181.41 ± 31.03	176.00 ± 31.11	0.236072
[mg/dL]	CG	179.00 ± 35.61	159.60 ± 14.28	168.00 ± 0.00	0.244641
LDL	PCOS	113.78 ± 33.12	97.94 ± 33.01	83.00 ± 0.00	0.146114
[mg/dL]	CG	97.21 ± 30.21	84.30 ± 19.50	62.00 ± 0.00	0.235718
HDL	PCOS	63.30 ± 17.99	57.70 ± 18.28	55.00 ± 0.00	0.470371
[mg/dL]	CG	65.15 ± 16.19	60.20 ± 13.68	93.00 ± 0.00	0.141430
TG	PCOS	102.69 ± 64.37	128.53 ± 101.77	76.00 ± 5.66	0.335585
[mg/dL]	CG	79.91 ± 52.06	75.20 ± 24.16	65.00 ± 0.00	0.923159

Table VI. *Anthropometric and metabolic variables according to the rs6454674 CNR1 polymorphism*
Tabela VI. *Antropometryczne i metaboliczne zmienne w zależności od polimorfizmu rs6454674 CNR1*

		T/T	G/T	G/G	p
BMI [kg/m^2]	PCOS	26.45 ± 6.68	27.72 ± 7.90	26.67 ± 6.45	0.708616
	CG	25.03 ± 6.54	25.28 ± 5.58	23.00 ± 3.84	0.870233
WHR	PCOS	0.82 ± 0.07	0.81 ± 0.08	0.81 ± 0.09	0.680679
	CG	0.79 ± 0.05	0.81 ± 0.07	0.77 ± 0.07	0.351029
Glucose mean	PCOS	85.78 ± 7.67	87.55 ± 11.13	88.15 ± 8.79	0.587216
[mg/dL]	CG	81.32 ± 8.31	80.10 ± 8.11	81.00 ± 5.66	0.854864
Insulin mean	PCOS	9.52 ± 6.54	11.38 ± 9.84	11.84 ± 8.08	0.489187
[μIU/mL]	CG	7.10 ± 5.21	9.85 ± 6.43	4.95 ± 5.99	0.167315
HOMA	PCOS	2.04 ± 1.46	2.42 ± 2.27	2.59 ± 1.74	0.519759
	CG	1.42 ± 1.06	2.00 ± 1.42	0.96 ± 0.74	0.179625
TC	PCOS	196.02 ± 34.75	193.40 ± 42.05	194.77 ± 31.96	0.950608
[mg/dL]	CG	173.04 ± 29.86	177.35 ± 37.10	176.50 ± 19.09	0.893056
LDL	PCOS	111.80 ± 31.88	113.30 ± 36.04	110.92 ± 35.55	0.969192
[mg/dL]	CG	91.84 ± 30.71	96.42 ± 28.38	94.50 ± 28.93	0.845473
HDL	PCOS	63.59 ± 18.03	59.89 ± 19.76	62.54 ± 17.36	0.665642
[mg/dL]	CG	66.16 ± 17.44	63.58 ± 15.55	66.00 ± 16.09	0.836902
TG	PCOS	106.47 ± 80.17	116.05 ± 75.77	102.31 ± 41.82	0.785636
[mg/dL]	CG	74.68 ± 34.56	82.10 ± 57.89	80.50 ± 19.09	0.850169

Table VII. *Anthropometric and metabolic variables according to the rs2023239 CNR1 polymorphism*
Tabela VII. *Antropometryczne i metaboliczne zmienne w zależności od polimorfizmu rs2023239 CNR1*

	T/T		C/T	C/C	p
BMI [kg/m^2]	PCOS	26.90 ± 7.38	28.01 ± 7.31	24.48 ± 5.30	0.568435
	CG	24.4 ± 5.09	26.98 ± 7.33	19.29 ± 0.00	0.204006
WHR	PCOS	0.82 ± 0.07	0.83 ± 0.09	0.78 ± 0.07	0.254993
	CG	0.80 ± 0.06	0.81 ± 0.06	0.81 ± 0.00	0.823443
Glucose mean	PCOS	86.10 ± 10.33	88.03 ± 8.91	85.73 ± 6.83	0.747082
[mg/dL]	CG	81.07 ± 8.20	79.59 ± 8.05	82.00 ± 0.00	0.809505
Insulin mean	PCOS	9.48 ± 6.16	12.25 ± 10.93	11.41 ± 6.94	0.476242
[μIU/mL]	CG	8.16 ± 5.81	9.66 ± 6.61	5.10 ± 0.00	0.598160
HOMA	PCOS	2.00 ± 1.45	2.69 ± 2.41	2.43 ± 1.55	0.401481
	CG	1,68 ± 1.30	1.87 ± 1.31	1.03 ± 0.00	0.770856
TC	PCOS	202.57 ± 38.49	181.50 ± 30.03	179.09 ± 30.11	0.028073
[mg/dL]	CG	176.47 ± 36.20	173.53 ± 27.43	168.00 ± 0.00	0.932694
LDL	PCOS	118.07 ± 32.42	99.60 ± 32.57	101.60 ± 33.89	0.059394
[mg/dL]	CG	95.55 ± 30.56	93.53 ± 25.20	62.00 ± 0.00	0.521619
HDL	PCOS	64.08 ± 18.80	58.33 ± 17.58	60.00 ± 15.81	0.533827
[mg/dL]	CG	65.47 ± 16.37	61.47 ± 14.35	93.00 ± 0.00	0.144478
TG	PCOS	109.10 ± 69.85	117.57 ± 82.22	80.00 ± 61.64	0.473616
[mg/dL]	CG	73.50 ± 46.38	92.24 ± 51.26	65.00 ± 0.00	0.390350

is related to various metabolic disturbances, we investigated the effect of six genetic variations in the CNR1 gene on anthropometric and metabolic parameters in 130 women with PCOS.

Our study suggests no associations between the CNR1 polymorphisms and BMI, WHR and various metabolic variables (glucose, insulin, HOMA-IR and lipids) in PCOS patients. Only women carrying the T/T genotype of rs2023239 CNR1 polymorphism had higher total cholesterol and LDL cholesterol levels than those with C/T and C/C variants. No correlations were found between the other studied CNR1 polymorphisms and anthropometric and metabolic parameters in women with PCOS. It is worth noting that studied women were young (24.91 ± 4.85 years) and were not obese, but were overweight (BMI 26.96 ± 6.96 kg/m^2). Maybe if only obese women had been studied, the results would have been different.

To date there have been no studies of CNR1 polymorphism in women with PCOS. Similar to our results, but in other groups of subjects (German children and adolescents), Müller et al. did not find any relation between rs2023239 variants and obesity [23]. Lieb et al. also found no evidence of an association of CNR1 genotypes and parameters related to obesity [24]. Zhuang et al. investigated rs2023239 and rs806381 polymorphisms in Chinese retired women and concluded that these variants are not associated with increased overweight and obesity risk [25]. In another study, de Luis et al. detected no differences in anthropometric parameters in patients with obesity and diabetes mellitus type 2 and rs1049353 CNR1 polymorphism [26]. In our previous studies, we did not observe relations between the A3813G (rs12720071), G1422A (rs1049353), A4895G (rs806368), rs806381, rs10485170, rs6454674 and rs2023239 CNR1 polymorphisms and anthropometric measures, or carbohydrate and lipid metabolism in postmenopausal women. On the other hand, we noted significant associations between the 3813G allele and higher android fat deposit and percentage of android fat [22, 30].

In contrast to these findings, some studies revealed an association between various genetic variants of the CNR1 gene and obesity. Peeters et al. observed a relation between rs1049353 polymorphism (A/A genotype) and higher WHR and waist circumference [21]. Russo et al. found that subjects with the 12720071G allele had higher waist circumference and subscapular skinfold thickness [20]. In other studies, Jaeger et al. revealed an association between the rs806368G allele and WHR [19], and Benzinou et al. reported a relation between rs2023239 and rs806381 CNR1 variants and BMI [31]. Hu et al. showed that subjects with metabolic syndrome and G/A and A/A genotypes of G1359A CNR1 polymor-

phism had lower BMI, waist circumference, HOMA and triglycerides [32]. The same results were demonstrated by Liu et al. in patients with coronary artery disease [33]. Similarly, Wang et al. found a correlation between G/G genotype of G1359A and increased values of BMI, HOMA and decreased HDL cholesterol level [34].

On the basis of previous studies, as yet there is no single, agreed answer to the question as to the relation between CNR1 polymorphism and obesity and related metabolic disturbances. The different results of the abovementioned studies may have resulted from genetic and phenotypic heterogeneity and differences of the studied population (men, women, obese and lean people, healthy and with various diseases and syndromes, various nationality, various age of subjects). Environmental factors, the kind of diet, physical activity and chronic stress may also influence the results of studies in CNR1 polymorphism.

In conclusion, on the basis of our study, it seems that CNR1 polymorphisms are not associated with obesity and metabolic disorders, including insulin resistance, in PCOS women. Further investigations in a larger population of women with PCOS need to be performed.

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